Investigation of the role of Selected Probiotics in Degradation of 4-Nitroquinoline-1-oxide,1,2- Dimethylhydrazine and Azoxymethane Food Derivatives

Synopsis

Submitted in the Partial Fulfillment for the Requirement for

Doctor of Philosophy

In

Botany

Submitted By

Priyanka Chaturvedi

Under the Supervision of

Dr. Prem Saran Tirumalai

Assistant Professor

Department Of Botany

DEPARTMENT OF BOTANY, FACULTY OF SCIENCE

DAYALBAGH EDUCATIONAL INSTITUTE (DEEMED TO BE UNIVERSITY)

DAYALBAGH, AGRA-282005

2022

TABLE OF CONTENTS

S.no	TITLE	Pg. No
1.0	**INTRODUCTION**	**1**
2.0	**REVIEW OF LITREATURE**	**6**
	Probiotics and its uses in human health	
	Polycyclic Aromatic Hydrocarbons (PAH's)	
	Act as Carcinogens	
	Carcinogens Involved in Colorectal Cancer	
	Probiotics helps in Degradation of Carcinogens	
	Probable use of Probiotics in Cancer Therapy	
3.0	**OBJECTIVES**	**13**
4.0	**PLAN OF STUDY AND METHADOLOGY**	**13**
5.0	**RATIONALE**	**16**
6.0	**EXPECTED OUTCOME**	**16**
7.0	**REFRENCES**	**16**

LIST OF TABLES

T.NO	TITLE	Pg No
1.0	No of Cases per year of different types of cancer	2
2.0	Types of Carcinogens responsible for causing cancer	3
3.0	List of some health benefit of *Lactobacillus plantarum*	12

1.0 INTRODUCTION

1.1 Food consumption and Lifestyle

According to World Health Organization (WHO) 2020, Healthy diet means consuming the healthy food throughout the life-course helps to prevent malnutrition in all its forms as well as the range of non-communicable diseases and conditions. However rapid urbanization, increased production of processed food and changing lifestyle have led to a shift in dietary pattern. In addition to the biological functioning of the eating, it is also considered to be principle social and cultural activity that people tend to enjoy for aesthetic and communal reasons (Cornil & Chandon, 2016). However, it is no longer a sole source of pleasure and enjoyment nowadays, but has increasingly become a cause of concern because of its potential consequences of ill health. Food habits is one factor that is most consistently linked to eating behavior (Verplanken, 2006). Many researches have been shown that Habits are strong predictors of eating behaviour (Gardner, 2011; Van't Riet, Sijtsema, Dagevos, & De Bruijn, 2011). In fact, later it is concluded that habit alone can explain about 20% variation in nutritional related behaviors (Gardener *et al.*, 2011).

1.2 Lifestyle Problems Related to Food Consumption

1.2.1 Inflammatory Bowel Diseases (IBD)

Inflammatory Bowel Disease (IBD) is a chronic inflammatory disorder of gastrointestinal tract which is thought to activate the intestinal mucosal immune system in response to the dysbiosis in the gastrointestinal tract in genetically susceptible person (Kovacs *et al*.,2012)). Diet plays an important role in altering the microbiome and modifies the intestinal immune response and so could play a role in pathogenesis of IBD (Brown *et al*., 2012). There is an increase in the incidence rate of IBD in several countries and this increase is because of the adoption of western diet.

Dietary changes including increased intake of refined sugars and dietary fats and decreased intake of fiber, have been suggested to be the potential risk factor for the development of IBD (Racine *et al*., 2016, Ananthakrishnan *et al*., 2013).

Gastroparesis were most commonly found in Inflammatory Bowel Syndrome (IBS) patients with dyspepsia. Other researchers suggest that between 23% to 87% of IBS patients have functional dyspepsia and between 13% to 87% of patients with dyspepsia have IBS, therefore it suggests that

gastroparesis symptoms are common in IBS patients, but in most cases it appears in IBS patients with dyspepsia (Stanghellini *et al.*, 2002 and Frissora *et al*., 2005).

1.2.2 Diabetes

There are many possibilities that food consumption can cause Type 2 Diabetes. Many discoveries shown that intake of potatoes, red meat and processed meat has been positively related to the risk of type- 2 diabetes (Ylonen *et al*., 2007). However, a study from Italy demonstrated that in obese (not in lean), insulin-resistant women, consumption of food fried in extra-virgin olive oil significantly reduced both insulin and C-peptide responses after a meal (Farnetti *et al*., 2011).

1.2.3 Food Relation with Obesity

Several studies reported a positive association between fried food intake and being overweight waist circumference or weight gain among pregnant women (Stuebe *et al*.,2009). More research is needed to quantify the impact of the type of oils used for frying and the frying conditions on the development of obesity.

1.2.4 Food Relation with Heart disease

Cardiovascular Diseases (CVD) is the leading global cause of death and is projected to be associated with more than 23.6 million deaths by 2030 (Mozaffarian, *et al*., 2015).

1.3 Cancer due to Food and Eating habits

Cancer is a large group of diseases that can affect various parts of the body. According to the World Health Organization (WHO) One defining feature of cancer is rapid formation of abnormal cells that grow beyond their usual boundaries, and which can invade the adjoining parts of the body and spread to other organs, the latter process is referred to as metastasis. Metastasis is the primary cause of death from cancer (World Health Organization, 2021).

Cancer is one of the leading causes of death worldwide, accounting for nearly 10 million death in 2020.

WHO listed the most common types of cancer cases occurred in 2020. (Table 1)

TABLE 1 No of Cases per year of different types of cancer

S.No	Types of cancers	No. of cases/year 2020
1	Breast Cancer	2.26 million
2	Lung Cancer	2.21 million
3	Colon and Rectum Cancer	1.93 million
4	Prostate Cancer	1.41 million
5	Skin Cancer	1.20 million
6	Stomach Cancer	1.09 million

(Source:WHOhttps://www.who.int/newsroom/factsheets/detail/cancer)

According to the estimates of the World Health Organization (WHO), 2019 cancer is the first or second leading cause of death before the age of 70 years in 112 out of 183 countries and rank third or fourth in a further 23 countries (WHO,2020). In contrast to other regions, the share of cancer deaths in Asia (58.3%) and Africa (7.2%) are higher than the share of incidence (49.3% and 5.7%, respectively) because of the different distribution of cancer types and higher case fatality rates in these regions.

Here are types of Carcinogens which are responsible for causing cancer are listed below-

Table 2- Types of Carcinogens responsible of causing cancer

Column1	Column2
Physical Carcinogen	Ultravoilet and ionizing radiation
Chemical Carcinogen	Abestos, components of tobacco smoke
Biological Carcinogen	Infections from certain viruses,bacteria or parasites

(Source : WHO https://www.who.int/news-room/fact-sheets/detail/cancer)

1.3.1 Physical Carcinogen

Physical Carcinogens comprises of exposure to ionizing radiations like X-rays, ultraviolet rays and gamma rays, or the environment which showed the formation of high energy secondary electrons, these interactions can directly or indirectly affect the DNA strand and it eventually leads to cancer.

Such exposure can cause:

- Formation of pyrimidine dimer
- Apurinic sites with a consequent break in DNA
- Formation of free radicals and superoxide, which cause DNA break, leading to somatic mutations. (Islam M, 2006).

1.3.2 Chemical Carcinogen

Chemical carcinogens are the compounds that can become a precursor when they are established in our body via occupation, lifestyle or diet. Cigarette smoking is a lifestyle hazards which is the main cause of lung cancer worldwide. Potent carcinogens which are found in cigarettes smoke includes Polycyclic Aromatic Hydrocarbons, benzene and Nitrosamine. Other lifestyle hazards involve chewing tobacco that leads to mouth cancer, drinking alcohol which leads to mouth, pharyngeal, esophageal and liver cancer. Preservative such as nitrites which is used in storage of meat has been labelled as carcinogenic. (Bhatia, *et al*.,2020).

1.3.3 Biological Carcinogens

Some of the infectious agents that have been classified as group 1 carcinogens (carcinogenic to humans) are bacterium Helicobacter pylori viruses [Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human Papillomavirus (HPV), Epstein-Barr virus (EBV), Human T-cell lymphotropic virus type 1 (HTLV-1) and Human Immunodeficiency virus-1 (HIV-1)] and parasites (Schistosoma haematobium (SH), Opisthorchis viverrini (OV) and Clonorchis sinensis (CS) IARC,2012**.**

Monographs on the evaluation of carcinogenic risks to humans "a review of human carcinogens:."(https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5203929/).

1.3.4 Colorectal Cancer and Food

Some recent research proved that eating processed meat increases the risk of colorectal cancer. The World Cancer Research Fund (WCRF) has recently recommended that people should avoid eating processed meat. Processed meat is any kind of meat that has been preserved by curing, salting or smoking or by adding chemical preservatives. There is convincing evidence that red meat also increases a person's risk of colorectal cancer. Food that may increase cancer risk are as follows:

- **Artificial Sweeteners -** like Aspartame, Cyclamate and Saccharine.
- **Cured pickle and salty foods**- bacon and other cured and pickled meat contain a substance called nitrate, which has the potential to cause cancer, and in particular bowel cancer when eaten in large doses.
- **Burnt or barbecued foods**- A group of carcinogenic substances called polycyclic aromatic

hydrocarbons (PAH's) can be produced if food are overheated or burnt.

1.4 Probiotics and its Role in Food

Probiotics are the live microbial feed supplement which affects the host beneficially by improving its intestinal microbial balance, in other words Probiotics are the live microbial food ingredient that is beneficial to health. Probiotics is derived from a latin word (pro) and Greek word (bios) which means "for life". Probiotics have become popular in recent years because they are improving human health through nutrition.

Firstly, the concept of Probiotics was proposed by a Russian Scientist Ilya Ilyich Metchnikoff who suggested that the long healthy Bulgarian Peasants could be because they consume fermented milk products. In 1899 Henry Tissier first isolated Bifidobacteria from the stools of breast feed infants and they found that there was a predominant component of the intestinal flora in healthy humans and it was later recommended that the administration of Bifidobacteria is beneficial to infants with diarrhea. Probiotics plays an important role in either shortening the duration or prevention from infections. The consumption of Probiotics is in dairy-based foods form, which contain two bacterial species Lactobacillus and Bifidobacterium.

Lactobacilli are one of the fastidious Gram-positive bacteria that colonize in nutrient rich habitats associated with food, feed, plants, vertebrates and invertebrates animals and humans, therefore research has focused in their role in food fermentation and spoilage (G¨anzle and Ripari., 2016). Lactobacillus genus comprises 200 species which are characterized by a phylogenetic and metabolic diversity that exceeds that of a typical bacterial family (Sun *et al*., 2015).

Bifidobacterium genera represent one of the most prominent microbial groups that occur in the gut of various animals including warm-blooded animals and social insects (Ventura *et al*.,2015). Bifidobacteria reach particularly high relative abundance in the infant gut microbiota, and this prevalence support their role as modulators of various metabolic and immune activities of their host (Ventura *et al*., 2012). Currently, the genus Bifidobacterium includes 47 taxa, involving 38 species and 9 subspecies (Ventura *et al.*, 2014; Biavati *et al*.,1991; Endo *et al*.,2012). However, representatives of only 10 of the 47 currently recognized Bifidobacterial sub-species have been genomically decoded (Ventura *et al*., 2012).

2.0 REVIEW OF LITREATURE

2.1 Probiotics and its uses in human health

The human Gastrointestinal tract contains a complex and dynamic microbial ecosystem composed trillion of microorganism that are collectively known as gut microbiota. Important advances have shown that several highly prevalent health disorders, including inflammatory bowel disease (IBD) and acute infectious diarrhea, and chronic diseases such as type-2 diabetes and obesity, are associated with the altered microbiota composition as well as imbalance host microbiota interactions (Liang *et al* ., 2018).

2.1.1 Probiotics in the treatment of Gastrointestinal disorder

Many clinical evidences shown that consumption of dairy products containing Bifidobacterium longum BB536, including yogurts, yogurt drinks, and non-fermented milks, can improve the density of defecation and fecal characteristic in healthy adults with constipation (Ogata *et al*., 1997, 1999; Xiao, Kondo, Odamaki, *et al*., 2007; Yaeshima *et al.*, 1997, 1998, 2001).

2.1.2 Probiotics used as Antiallergic

Seasonal allergic rhinitis caused by Japanese cedar pollen is one of the most prevalent forms of allergic disease in Japan and is considered a national affliction (Yamada *et al*., 2014). Another double-blind, placebo-controlled, randomized trial involving 40 Italian children (4–17 years) treated with probiotics mixture containing three bifidobacterial strains, BB536 (3×109 CFU), B.longum subsp. infantis M-63 (1×109 CFU), and B. breve M-16V (1×109 CFU), for 4 weeks has also revealed a significant improvement in pollen-induced allergic rhinitis (Del Giudice *et al*., 2017).

2.1.3 Probiotics used in Lactose Intolerant Condition

Lactose intolerant condition (LI) is a condition characterized by inability to digest lactose due to low level of lactase enzyme activity. Over 30 million people have some degree of LI by age 20 in USA. Lactase activity declines with age, resulting in unabsorbed lactose being metabolized by colonic bacteria to produce short chain fatty acids and gas (Mattar *et al.,* 2012). Probiotics promote lactose digestion in LI by increasing the overall hydrolytic capacity in the small intestine and increasing the colonic fermentation (Dhama *et al*., 2016).

The consumption of Probiotics has been shown to enhance health benefits by preventing chronic

degenerative diseases (Obesity, diabetes, cancer and heart disease) (Abdelazez *et al*., 2017).

Lactobacillus plantarum is easy to be cultured, therefore it is highly in demand by researchers in food industry application since it considered to be safe probiotic (Zago *et al*., 2011).

Due to potential teratogenic effects of common prescription, over-the-counter, and supplementary medications on organogenesis and embryonic development, the use of probiotics during pregnancy has become a topic of increasing interest for peripartum healthcare professionals and new mothers alike (Genuis et al., 2016 and Alwan *et al.*, 2015).

2.1.4. a. *Lactobacillus casei* characteristics in general

Lactobacillus was firstly described in 1901 with Lactobacillus delbrueckii, an indispensable species in yogurt productions. In 1935, Japanese researchers Dr.Shirota discovered the strain Lactobacillus casei Shirota with proven health benefit and it was the first commercial product which was sold for the first time in 1995 in Japan (Yerlikaya, 2014).

Therefore, in recent years numerous scientific genetic analyzes show an enormous heterogeneity among the more than 260 species of the genus Lactobacillus ((Park *et al*., 2018; Salvetti *et al*., 2018). Lactobacillus casei is one of the most studied species due to its industrial, commercial and applied health benefit ((Hill *et al*., 2018; Zheng *et al*., 2020). Particularly, L. casei-01 strain has been traditionally used as probiotic culture in dairy products ((Balthazar *et al.*, 2018; Costa, Paula, *et al*., 2019; Zheng *et al*., 2020).

2.1.5.a. Lactobacillus plantarum characteristic in general

Lactobacillus plantarum is a facultative homofermentative lactic acid bacterium which is dispersed in the nature too a great extent (Siezen *et al*., 2010). L. plantarum is mainly found in the human and other mammalian gastrointestinal tracts, saliva and various food products, it is Gram positive, rod shaped, catalase negative and non- sporing bacteria (Holzapfel *et al*., 2001). Thus, it can adapt to varied of different environments. Moreover, it has the biggest genome (~ 3.3Mb) among the Lactic Acid Bacteria group and it can be isolated from various sources (Martino *et al*., 2016). It is extremely tolerant to various harsh conditions including the digestive system stresses (Parente *et al*., 2010), gastrointestinal, vaginal and urogenital tracts (Jose *et* al., 2015).

Lactobacillus plantarum is very easy to be cultured therefore it is highly interested by the researchers

in the food industry application since it is considered to be the safe probiotic (Zago *et al.*, 2011). L. plantarum is widely used in the pharma industries therefore it is significantly used in human medicine without causing any side effects and this LAB has been broadly used as a live diet supplement food industry, and in some recent research it has been reported that it could be used as a vaccine vehicle (Quatravaux et al., 2006).

2.1.5.b. Health benefit of *Lactobacillus plantarum* in general

Table - 3 List of some health benefit of *Lactobacillus plantarum*

Immune-modulatory effects	Ku *et al.* , 2014
Lowering cholesterol	Li *et al*., 2014
Irritable bowel syndrome	Stevenson *et al.* , 2014
Plaque inflammation	Kim *et al* ., 2013
Crohn's disease and ulcerative colitis	Chiu *et al.* , 2013

2.1.6.a. *Bifidobacterium bifidum* characteristic in general

Bifidobacterium bifidum is a gram-positive bacteria with a high Guanine and Adenine DNA content, and it was first isolated from feces of breast fed infants by Tissier in 1899 and then named *Bacillus bifidus* (Tissier,1990). Since it was morphologically and physiologically similar to those of lactobacilli, they were classified as member of the genus Lactobacillus for most of the 20th century and from 1974 it has been recognized as a separate genus (Buchanan, 2009).

Bifidobacterium bifidum are specifically identified in the human gut and have been shown to represent part of the dominant bacterial members of the gut- microbiota of breast feed infants (Turroni *et al.*, 2009,2012). The genome size of a *B. bifidum* ranges from 2.14 to 2.28 Mb (Ventura *et al*., 2007b, 2012; Turroni *et al.*, 2013b).

2.1.6.b. Health benefit of Bifidobacterium bifidum in general

- Antibacterial activities against *Helicobacter pylori* - (Shirasawa *et al.*, 2010; Chenoll *et al*., 2011).
- Reduction in the intestinal epithelium of infants suffering from necrotising enterocolitis - (Khailova *et al* , 2010).
- Alleviation of anti-inflammatory activities associated with certain chronic large bowel dysfunction - (Mouni *et al*., 2009; Guglielmetti *et al*., 2011).
- Helpful in the modulation of the host-immune system - (Fu *et al*., 2010; Philippe *et al.*, 2011).

2.2 Polycyclic Aromatic Hydrocarbons (PAH's) Act as Carcinogens

2.2.1 PAH's present in Processed Food

Polycyclic Aromatic Hydrocarbon (PAH's) are a structurally similar chemical compounds characterized by fused aromatic rings. (PAH's) may form directly in food as a result of some heat process like charcoal grilling, roasting and smoke drying as intense thermal process can be applied to foods in direct and indirect way (Kumosani *et al*.,2013). Grilled meat in general estimated to contain around 10.5 n/g benzopyrene (Jaegerstad *et al*., 2005). It was found that the highest concentrations of PAHs were observed in pizza baked in wood-burning ovens and barbecued beef and pork (Lodovici *et al*.,1995). The usage of nitrate and nitrite in food preservation, were nearly banned from use in food in 1970 due to data suggesting possible health concern and nitrites remains the most feared by consumers. As stated previously, nowadays the consumption of red meat has been linked to the increased incidence of CRC, also the cancer of esophagus, gastric cancer, liver cancer and bladder cancer (Zhu *et al*., 2014). On the contrary, poultry and fish intake has been associated with lower risk of digestive and respiratory cancers (Daniel *et al*., 2011a).

2.3 Carcinogens Involved in Colorectal Cancer

Alimentary factors may have major role in colorectal cancer and highest incidence rate noted in countries where the diet mainly consist of red processed meat with low amount of fibre sources.

Processing of red meat especially frying or grilling at high temperature, causes degradation of molecule Creatinine and amino acids, resulting in the formation of numerous carcinogenic heterocyclic amines (Sugimura *et al.,* 2004 and Martinez *et al.,* 2007).

2.3.1 4- Nitroquinoline-1-oxide as a potential carcinogen responsible for Colorectal Cancer

4-Nitroquinoline-1-oxide (4NQO), is a quinoline, a carcinogenic and mutagenic chemical. It possesses a heterocyclic aromatic structure, which basic chemical formula of C9H7N. 4-NQO may naturally occur in the environment but it is typically manufactured for research purpose (La Voie *et al*., 1983). The reduced metabolite of 4-NQO known as 4- hydroxy amino quinoline 1-oxide (4-HAQO) bind covalently to cellular macromolecule such as nucleic acid and proteins (Tada *et al*.,1975). 4-NQO induces DNA damage through the production of reactive species which is thought

to arise from enzymatic reduction of its nitro group, although the exact mechanism is still unknown (Arima *et al*.,2006).

2.3.2 4-NQO responsible for carcinogenesis

4-NQO considered to be a tobacco-mimicking carcinogen that induces intracellular oxidative stress, resulting in the generation of the reactive oxygen species (ROS) metabolic product that bind to DNA and cause DNA adducts (Nagao & Sugimura, 1976), therefore this carcinogen classified as a DNA reactive genotoxin (Miranda *et al.*, 2011).

2.3.3 1,2- Dimethylhydrazine as carcinogen

1,2- Dimethylhydrazine is the organic compound with the formula (CH3NH)2, it is one of the two isomers of dimethylhydrazine and these both isomers are colorless at room temperature, with properties similar to those of methylamines. 1,2- DMH considered to be the potent carcinogen that act as a DNA methylating agent, it has no commercial value, in contrast to its isomers, which is used as a rocket fuel (Chodhary *et al*.,1998). It is used to induce colon tumors in experimental animals-particularly mice and feline cell samples (Cruse *et al*., 1978 and Wijnands *et al*., 1999).

2.3.4. 1,2- Dimethylhydrazine (DMH) in relevance to the Colon Cancer

The organotrophic colon carcinogen DMH is an alkylating agent widely used to induce benign and malignant neoplasm in the colon of rodents (Cooper *et al.*, 1978). In some animal studies, repeated exposure of DMH produces colon tumors, which shows the pathological features which are similar to the sporadic forms of human colon cancer (Maskens., 1976).

2.3.4 Azoxymethane as carcinogen

Azoxymethane is a potent carcinogenic agent commonly used to induce colon cancers in rats. The AOM induced colon carcinogenesis model is frequently used in developing chemoprevention strategies (Al-Henhena N *et al*., 2014). In this study, the chemopreventive effect of juniper oil was investigated in the AOM-induced colon cancer model.AOM-induced mutations mostly result from direct DNA-carcinogen interactions, the mutagenic action of iron is believed to be indirect, namely, free radical-induced oxidative modification of DNA (Pallem *et al*.,2020).

2.4 Probiotics helps in Degradation of carcinogens

Lactobacillus rhamnosus (IMC501) characterized for its protective effects against genotoxic

damage induced in mice by food mutagen 2-amino-1-methyl-6-phenylimidazo (4,5-b) pyridine (PhIP) was used (Domnici L *et al*., 2014).

Some studies showed that *Bifidobacterium pseudocatenulatum* G4, *Bifidobacterium longum* and *Escherichia coli* shown binding of mutagens Trp-p-2, IQ, MeIQx, 7,8DiMeIQx and PhIP at different pH was analysed by using HPLC technique.

Prebiotic supplementation independently or with probiotics (*Lactobacillus rhamnosus GG* and *Bifidobacterium lactis* Bb12) helps to produce protective effects against azoxymethane- induced experimental colon cancer (Femia *et al*., 2002) as well as it helps in reducing the activity of nitric oxide synthetase and cyclooxygenase-2 which are usually elevated during colon cancer (Ahn *et al*., 2001).

Some studies revealed that probiotic bacteria such as *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Lactobacillus salivarius* protect against preneoplastic lesions, including aberrant crypt foci, mucin depleted foci and also modulate colonic microflora structure and improve the luminal metabolism in 1,2- dimethylhydrazine induced colorectal carcinogenesis in rats (Mohania *et al*.,2014).

Also, one report suggest that these probiotic species protect the gut against the formation of precancerous lesions by suppressing the activity of carcinogen enzyme such as Azoreductase (Burns *et al.*, 2000). Several studies suggest that the changes in the number or/and composition of their population are one of the most frequent situations that present in some diseases like inflammatory bowel diseases, colorectal cancer or irritable bowel syndrome (Tojo *et al.*, 2014). The administration of *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *B.infantum* altered the gut microbiota and decreased colon cancer development by decreasing tumor incidence, multiplicity/count and volume (Kuugbee *et al.*, 2016).

2.4 Probable use of Probiotics in Cancer Therapy

In recent studies states that the probiotics have been demonstrated to confer beneficial effects on colon cancer because of the direct exposure of the colon to consumed bacteria (Ranjbar *et al.*, 2019).

The study suggest that Probiotics may act as antimutagens and exert constructive effects 17 at different stages of carcinogenesis. The anticancer effects of 18 probiotics could be attributed to the following: (Raman *et al*, 2013)

- ❖ Inactivation of mutagens or carcinogens,
- ❖ Immunomodulatory effects,
- ❖ Intestinal microflora modulation,
- ❖ Modulation of apoptosis and cell differentiation, and
- ❖ Tyrosine kinase signaling pathway inhibition

Potential probiotic human strains include *L. rhamnosus* 231 bind 3 methyl nitronitroso guanidine (MNNG) and 2-amino-3,4-dimethyl4 imidazo[4,5-f]-quinoxaline (MeIQx) leading to biotransformation, 5 detoxification and thus, exhibit antimutagenic activities (Ambalam *et al.*, 2011). Some studies also showed that the antimutagenic and antigenotoxic 7 activities of *Lactobacillus rhamnosus* Vc against MNNG. Hence, it 8 could be contemplated that probiotic bind and assist in the 9 metabolic excretions of mutagens (Pithva *et al.*, 2016).

3.0 OBJECTIVES

1. To study the effect of *Lactobacillus casei* on selected food derived carcinogens (4-Nitroquinoline-1-Oxide,1,2- Dimethylhydrazine and Azoxymethane.)
2. To study the effect of *Lactobacillus plantarum* on selected food derived carcinogens. (4-Nitroquinoline-1-Oxide,1,2- Dimethylhydrazine and Azoxymethane.)
3. To study the effect of *Bifidobacterium bifidum* on selected food derived carcinogens. (4-Nitroquinoline-1-Oxide,1,2- Dimethylhydrazine and Azoxymethane.)
4. To study the effect of consortia of the selected probiotic species on the group of food derived carcinogens (4-Nitroquinoline-1-Oxide,1,2- Dimethylhydrazine and Azoxymethane.)

4.0 Plan of Study and Methodology

1. Culture Procurement

Cultures are procured by some Standard National and International Repositories.

- *Lactobacillus plantarum* and *Bifidobacterium bifidu*m are procured by Agricultural Research

Services (ARS) Northern Regional Research Laboratory (NRRL), United States Department of Agriculture (USDA), Peoria, USA. and

- *Lactobacillus casei* are procured by National Collection of Industrial Microbes (NCIM), Pune, INDIA.

2. Culture Preservation and Maintenance

Cultures are preserved by following methods:

- Glycerol Stock solution : Glycerol Stock Solution are used to preserve the culture. 50 microlitre of is used for the isolation of pure culture of bacteria. The inoculum is streaked over the agar surface 50% of glycerol solution will be mixed with 50 microlitre of bacterial culture in Eppendorf tubes and preserve this stock solution will be feezed at -70 degrees for 3-4 months. (Deutz *et al*,. 2000)
- Slant cultures : In this method the culture is streaked over the agar slant to view and identify colonial characteristics, and also provide a way to separate bacteria.
- Plate Culture Method - This method will be used to preserve the cultures. Streak plate technique in such a way that individual bacterial cells are separated and well - spaced from each other.

3. To Investigate the effect of selected Food derived carcinogens on selected Probiotic Species.

- Effect of food derived carcinogen (4- Nitroquinoline-1-Oxide) on *Lactobacillus casei, Lactobacillus plantarum and Bifidobacterium bifidum* will be studied.
- Effect of food derived carcinogen (1,2- Dimethylhydrazine) on *Lactobacillus casei, Lactobacillus plantarum and Bifidobacterium bifidum* will be studied.
- Effect of food derived carcinogen (Azoxymethane.) on *Lactobacillus casei, Lactobacillus plantarum and Bifidobacterium bifidum* will be studied.
- Inoculation – The cultures will be inoculated in a broth medium with or without carcinogens and will be kept for 0h, 24h, 48h and 72h.
- Serial dilution and Standard Plate Count- Then cultures will be serially diluted in Saline Solution and plates will be incubated for 48 h and then the growth will be observed and expressed in CFU/ml.

4. To detect the amount of degradation of selected Carcinogens by using probiotic species

- High Performance Liquid Chromatography (HPLC) (Kim *etal*.,2010) is going to used to detect

the degradation of selected carcinogens by using selected probiotic species. To examine the effect of selected probiotic species on selected carcinogens the High Performance Liquid Chromatography (HPLC) will be used. It is an significant methodical method usually used to separate and quantify components of samples. In this technique, a solution (mobile phase) is pumped through a column (stationary Phase) that contains a packaging of small porous particle with a second phase bound to the surface.

- Effect of *Lactobacillus casei,* on (4 Nitroquinole-1-oxide,1,2-Dimethylhydrazine and Azoxymethane) using HPLC.
- Effect of *Lactobacillus plantarum,* on (4 Nitroquinole-1-oxide,1,2-Dimethylhydrazine and Azoxymethane) using HPLC.
- Effect of *Bifidobacterium bifidum,* on (4 Nitroquinole-1-oxide,1,2-Dimethylhydrazine and Azoxymethane) using HPLC.
- Effect of consortia of the selected species (*Lactobacillus plantarum* , *Lactobacillus casei* and *Bifidobacteria bifidum*) on (4 Nitroquinole - 1- oxide, 1,2- Dimethylhydrazine and Azoxymethane) using HPLC.

5.0 Rationale to the Study

Probiotics have been known to have a variety of health benefits, so much so that probiotics have been put to use in therapeutics. Can probiotics offer solutions in Cancer mitigation? Colorectal cancer which has been ranked as one among the most common cancers globally could be caused due to consumption of processed food. Processed food is a mixture of nutritional components but some practices are done to enhance the taste and texture of food. The Food undergo several changes during these practices and form Polycyclic Aromatic Hydrocarbons and Heterocyclic Amines. Can Probiotics be able to degrade these components?

6.0 Expected Outcome

The findings of this study will provide details on probable use of probiotics in colorectal cancer mitigation.

7.0 Refrences

1. World Health Organization (WHO). 2020. Cancer. https://www.who.int/news- room/fact-sheets/detail/cancer. Accessed at September 21, 2021.
2. Cornil, Y., & Chandon, P. (2016). Pleasure as an ally of healthy eating? Contrasting visceral and Epicurean eating pleasure and their association with portion size preferences and wellbeing. *Appetite*, *104*, 52-59.
3. Gardner, B., de Bruijn, G. J., & Lally, P. (2011). A systematic review and meta-analysis of applications of the self-report habit index to nutrition and physical activity behaviours. *Annals of Behavioral Medicine*, *42*(2), 174-187.
4. Onwezen, M. C., Van't Riet, J., Dagevos, H., Sijtsema, S. J., & Snoek, H. M. (2016). Snacking now or later? Individual differences in following intentions or habits explained by time perspective. *Appetite*, *107*, 144-151.
5. Brown, K., DeCoffe, D., Molcan, E., & Gibson, D. L. (2012). Diet-induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. *Nutrients*, *4*(8), 1095- 1119.
6. Verplanken, B. (2006). Beyond frequency: Habit as mental construct. *British Journal of Social Psychology*, *45*(3), 639-656.
7. Willett, W. C. (1994). Diet and health: what should we eat?. *Science*, *264*(5158), 532-
8. World Health Organization (WHO). (2020). Global Health Estimates 2020: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2019. *World Health Organization.*
9. Kovacs, A., & Gophna, U. (2012). Intestinal Microbiota and Intestinal Disease: Inflammatory Bowel Diseases. In *Beneficial Microorganisms in Multicellular Life Forms* (pp. 223-230). Springer, Berlin, Heidelberg.
10. Liang, D., Leung, R. K. K., Guan, W., & Au, W. W. (2018). Involvement of gut microbiome in human health and disease: brief overview, knowledge gaps and research opportunities. *Gut pathogens*, *10*(1), 1-9.
11. Racine, A., Carbonnel, F., Chan, S. S., Hart, A. R., Bueno-de-Mesquita, H. B., Oldenburg, B., ... & Boutron-Ruault, M. C. (2016). Dietary patterns and risk of inflammatory bowel disease in Europe: results from the EPIC study. *Inflammatory bowel diseases*, *22*(2), 345-354.

12. Tamaki, H., Nakase, H., Inoue, S., Kawanami, C., Itani, T., Ohana, M., ... & Shibatouge, M. (2016). Efficacy of probiotic treatment with Bifidobacterium longum 536 for induction of remission in active ulcerative colitis: A randomized, double-blinded, placebo-controlled multicenter trial. *Digestive Endoscopy*, *28*(1), 67-74.
13. Ogata, T., Nakamura, T., Anjitsu, K., Yaeshima, T., Takahashi, S., Fukuwatari, Y., ... & Iino, H. (1997). Effect of Bifidobacterium longum BB536 administration on the intestinal environment, defecation frequency and fecal characteristics of human volunteers. *Bioscience and Microflora*, *16*(2), 53-58.
14. Xiao, J., Kondo, S., Odamaki, T., Miyaji, K., Yaeshima, T., Iwatsuki, K., ... & Benno, Y. (2007). Effect of yogurt containing Bifidobacterium longum BB536 on the defecation frequency and fecal characteristics of healthy adults: A double-blind cross over study. *Jpn. J. Lact. Acid Bact*, *18*, 31-36.
15. Del Giudice, M. M., Indolfi, C., Capasso, M., Maiello, N., Decimo, F., & Ciprandi, G. (2017). Bifidobacterium mixture (B longum BB536, B infantis M-63, B breve M-16V) treatment in children with seasonal allergic rhinitis and intermittent asthma. *Italian journal of pediatrics*, *43*(1), 1-6.
16. Yamada, T., Saito, H., & Fujieda, S. (2014). Present state of Japanese cedar pollinosis: the national affliction. *Journal of Allergy and Clinical Immunology*, *133*(3), 632-639.
17. Mattar, R., de Campos Mazo, D. F., & Carrilho, F. J. (2012). Lactose intolerance: diagnosis, genetic, and clinical factors. *Clinical and experimental gastroenterology*, *5*, 113.
18. Dhama, K., K Latheef, S., K Munjal, A., Khandia, R., A Samad, H., MN Iqbal, H., & K Joshi, S. (2016). Probiotics in curing allergic and inflammatory conditions-research progress and futuristic vision. *Recent patents on inflammation & allergy drug discovery*, *10*(2), 105-118.
19. Khalili, H., Chan, S. S., Lochhead, P., Ananthakrishnan, A. N., Hart, A. R., & Chan, A. T. (2018). The role of diet in the aetiopathogenesis of inflammatory bowel disease. *Nature Reviews Gastroenterology & Hepatology*,
20. Islam, M. (2017). *Evaluation of Antioxidant Activity and Determination of Total Phenolic Content of leaves extract of Heritiera littoralis (Sundari tree)* (Doctoral dissertation, Daffodil

International University).

21. Frissora, C. L., & Koch, K. L. (2005). Symptom overlap and comorbidity of irritable bowel syndrome with other conditions. *Current gastroenterology reports*, *7*(4), 264-271.
22. Stanghellini, V., Tosetti, C., Barbara, G., De Giorgio, R., Cogliandro, L., Cogliandro, R., & Corinaldesi, R. (2002). Dyspeptic symptoms and gastric emptying in the irritable bowel syndrome. *The American journal of gastroenterology*, *97*(11), 2738-2743.
23. Jaegerstad, M., & Skog, K. (2005). Genotoxicity of heat-processed foods. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *574*(1-2), 156-172.
24. Kumosani, T. A., Moselhy, S. S., Asseri, A. M., & Asseri, A. H. (2013). Detection of polycyclic aromatic hydrocarbons in different types of processed foods. *Toxicology and industrial health*, *29*(3), 300-304.
25. Lodovici, M., Dolara, P., Casalini, C., Ciappellano, S., & Testolin, G. (1995). Polycyclic aromatic hydrocarbon contamination in the Italian diet. *Food Additives & Contaminants*, *12*(5), 703-713.
26. Alexander, D. D., Mink, P. J., Cushing, C. A., & Sceurman, B. (2010). A review and meta-analysis of prospective studies of red and processed meat intake and prostate cancer. *Nutrition journal*, *9*(1), 1-17.
27. Freedman, N. D., Cross, A. J., McGlynn, K. A., Abnet, C. C., Park, Y., Hollenbeck, A. R., ... & Sinha, R. (2010). Association of meat and fat intake with liver disease and hepatocellular carcinoma in the NIH-AARP cohort. *Journal of the National Cancer Institute*, *102*(17), 1354-1365.
28. Kim, E., Coelho, D., & Blachier, F. (2013). Review of the association between meat consumption and risk of colorectal cancer. *Nutrition research*, *33*(12), 983-994.
29. Zhu, H., Yang, X., Zhang, C., Zhu, C., Tao, G., Zhao, L., ... & Sun, X. (2013). Red and processed meat intake is associated with higher gastric cancer risk: a meta-analysis of epidemiological observational studies. *PloS one*, *8*(8), e70955.
30. Zhu, H. C., Yang, X., Xu, L. P., Zhao, L. J., Tao, G. Z., Zhang, C., ... & Sun, X. C. (2014). Meat consumption is associated with esophageal cancer risk in a meat-and cancer- histological-type

dependent manner. *Digestive diseases and sciences*, *59*(3), 664-673.

31. Daniel, C. R., Cross, A. J., Graubard, B. I., Hollenbeck, A. R., Park, Y., & Sinha, R. (2011). Prospective investigation of poultry and fish intake in relation to cancer risk. *Cancer prevention research*, *4*(11), 1903-1911.
32. Sun, Z., Harris, H. M., McCann, A., Guo, C., Argimón, S., Zhang, W., ... & O'Toole, P. W. (2015). Expanding the biotechnology potential of lactobacilli through comparative genomics of 213 strains and associated genera. *Nature communications*, *6*(1), 1-13.
33. Milani, C., Lugli, G. A., Duranti, S., Turroni, F., Mancabelli, L., Ferrario, C., ... & 34. Ventura, M. (2015). Bifidobacteria exhibit social behavior through carbohydrate resource sharing in the gut. *Scientific reports*, *5*(1), 1-14.
34. Sugimura, T., Wakabayashi, K., Nakagama, H., & Nagao, M. (2004). Heterocyclic amines: Mutagens/carcinogens produced during cooking of meat and fish. *Cancer science*, *95*(4), 290-299.
35. Martínez, M. E., Jacobs, E. T., Ashbeck, E. L., Sinha, R., Lance, P., Alberts, D. S., & Thompson, P. A. (2007). Meat intake, preparation methods, mutagens and colorectal adenoma recurrence. *Carcinogenesis*, *28*(9), 2019-2027.
36. LaVoie, E. J., Adams, E. A., Shigematsu, A., & Hoffman, D. (1983). On the metabolism of quinoline and isoquinoline: possible molecular basis for differences in biological activities. *Carcinogenesis*, *4*(9), 1169-1173.
37. Tada, M., & Tada, M. (1975). Seryl-tRNA synthetase and activation of the carcinogen 4-nitroquinoline 1-oxide. *Nature*, *255*(5508), 510-512.
38. Arima, Y., Nishigori, C., Takeuchi, T., Oka, S., Morimoto, K., Utani, A., & Miyachi, Y. (2006). 4-Nitroquinoline 1-oxide forms 8-hydroxydeoxyguanosine in human fibroblasts through reactive oxygen species. *Toxicological Sciences*, *91*(2), 382-392.
39. Turesky, R. J. (1994). DNA adducts of heterocyclic aromatic amines, arylazides and 4-nitroquinoline 1-oxide. *IARC scientific publications*, (125), 217-228.
40. Tang, X. H., Knudsen, B., Bemis, D., Tickoo, S., & Gudas, L. J. (2004). Oral cavity and esophageal carcinogenesis modeled in carcinogen-treated mice. *Clinical Cancer Research*,

10(1), 301-313.

41. Martínez, C. A. R. (2012). 4NQO carcinogenesis: A model of oral squamous cell carcinoma. *International Journal of Morphology*, *30*(1), 309-314.
42. Nagao, M., & Sugimura, T. (1976). Molecular biology of the carcinogen, 4- nitroquinoline 1-oxide. *Advances in cancer research*, *23*, 131-169.
43. Miranda, S. R., Noguti, J., Carvalho, J. G., Oshima, C. T. F., & Ribeiro, D. A.(2011). Oxidative DNA damage is a preliminary step during rat tongue carcinogenesis induced by 4-nitroquinoline 1-oxide. *Journal of molecular histology*, *42*(2), 181.
44. Lan, A., Li, W., Liu, Y., Xiong, Z., Zhang, X., Zhou, S., ... & Chen, X. L. (2016). Chemoprevention of oxidative stress-associated oral carcinogenesis by sulforaphane depends on NRF2 and the isothiocyanate moiety. *Oncotarget*, *7*(33), 53502.
45. Choudhary, G., & Hansen, H. (1998). Human health perspective of environmental exposure to hydrazines: A review. *Chemosphere*, *37*(5), 801-843.
46. Cruse, J. P., Lewin, M. R., Ferulano, G. P., & Clark, C. G. (1978). Co-carcinogenic effects of dietary cholesterol in experimental colon cancer. *Nature*, *276*(5690), 822-825.
47. Wijnands, M. V. W., Appel, M. J., Hollanders, V. M. H., & Woutersen, R. A. (1999). A comparison of the effects of dietary cellulose and fermentable galacto-oligosaccharide, in a rat model of colorectal carcinogenesis: fermentable fibre confers greater protection than non-fermentable fibre in both high and low fat backgrounds. *Carcinogenesis*, *20*(4), 651-656.
48. Cooper, H. K., Buecheler, J., & Kleihues, P. (1978). DNA alkylation in mice with genetically different susceptibility to 1, 2-dimethylhydrazine-induced colon carcinogenesis. *Cancer research*, *38*(9), 3063-3065.
49. Maskens, A. P. (1976). Histogenesis and growth pattern of 1, 2-dimethylhydrazine- induced rat colon adenocarcinoma. *Cancer research*, *36*(5), 1585-1592.
50. Abdelazez, A., Muhammad, Z., Zhang, Q. X., Zhu, Z. T., Abdelmotaal, H., Sami, R., & Meng, X. C. (2017). Production of a functional frozen yogurt fortified with bifidobacterium spp. *BioMed research international*, *2017*.
51. Zago, M., Fornasari, M. E., Carminati, D., Burns, P., Suàrez, V., Vinderola, G., ... & Giraffa, G.

(2011). Characterization and probiotic potential of Lactobacillus plantarum strains isolated from cheeses. *Food Microbiology*, *28*(5), 1033-1040.

52. González, L., Sandoval, H., Sacristán, N., Castro, J. M., Fresno, J. M., & Tornadijo, M. E. (2007). Identification of lactic acid bacteria isolated from Genestoso cheese throughout ripening and study of their antimicrobial activity. *Food Control*, *18*(6), 716-722.
53. Genuis, S. J., & Genuis, R. A. (2016). Preconception care: a new standard of care within maternal health services. *BioMed Research International*, *2016*.
54. Alwan, S., & Chambers, C. D. (2015). Identifying human teratogens: an update. *Journal of pediatric genetics*, *4*(02), 039-041.
55. Yerlikaya, O. (2014). Starter cultures used in probiotic dairy product preparation and popular probiotic dairy drinks. *Food Science and Technology*, *34*, 221-229.
56. Cabana, M. D., McKean, M., Caughey, A. B., Fong, L., Lynch, S., Wong, A., ... & Hilton, J. F. (2017). Early probiotic supplementation for eczema and asthma prevention: a randomized controlled trial. *Pediatrics*, *140*(3).
57. Park, S., Kim, J. I., Bae, J. Y., Yoo, K., Kim, H., Kim, I. H., ... & Lee, I. (2018). Effects of heat-killed Lactobacillus plantarum against influenza viruses in mice. *The Journal of Microbiology*, *56*(2), 145-149.
58. Salvetti, E., Harris, H. M., Felis, G. E., & O'Toole, P. W. (2018). Comparative genomics of the genus Lactobacillus reveals robust phylogroups that provide the basis for reclassification. *Applied and environmental microbiology*, *84*(17), e00993-18.
59. Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M., Harris, H., Mattarelli, P., ... & Lebeer, S. (2020). A taxonomic note on the genus Lactobacillus: Description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae.
60. Balthazar, C. F., Silva, H. L., Esmerino, E. A., Rocha, R. S., Moraes, J., Carmo, M. A., ... & Cruz, A. G. (2018). The addition of inulin and Lactobacillus casei 01 in sheep milk ice cream. *Food chemistry*, *246*, 464-472.
61. Costa, G. M., Paula, M. M., Barão, C. E., Klososki, S. J., Bonafé, E. G., Visentainer, J. V., ... &

Pimentel, T. C. (2019). Yoghurt added with Lactobacillus casei and sweetened with natural sweeteners and/or prebiotics: Implications on quality parameters and probiotic survival. *International Dairy Journal*, *97*, 139-148.

62. Hill, D., Sugrue, I., Tobin, C., Hill, C., Stanton, C., & Ross, R. P. (2018). The Lactobacillus casei group: history and health related applications. *Frontiers in microbiology*, *9*, 2107.
63. Zhang, G. Q., Hu, H. J., Liu, C. Y., Zhang, Q., Shakya, S., & Li, Z. Y. (2016). Probiotics for prevention of atopy and food hypersensitivity in early childhood: a PRISMA-compliant systematic review and meta-analysis of randomized controlled trials. *Medicine*, *95*(8).
64. Cabana, M. D., McKean, M., Caughey, A. B., Fong, L., Lynch, S., Wong, A., ... & Hilton, J. F. (2017). Early probiotic supplementation for eczema and asthma prevention: a randomized controlled trial. *Pediatrics*, *140*(3).
65. Psathas, J. N., Doonan, P. J., Raman, P., Freedman, B. D., Minn, A. J., & Thomas- Tikhonenko, A. (2013). The Myc-miR-17-92 axis amplifies B-cell receptor signaling via inhibition of ITIM proteins: a novel lymphomagenic feed-forward loop. *Blood, The Journal of the American Society of Hematology*, *122*(26), 4220-4229.
66. Dinan, T. G., & Cryan, J. F. (2017). The microbiome-gut-brain axis in health and disease. *Gastroenterology Clinics*, *46*(1), 77-89.
67. Dickerson, F., Adamos, M., Katsafanas, E., Khushalani, S., Origoni, A., Savage, C.,... & Yolken, R. H. (2018). Adjunctive probiotic microorganisms to prevent rehospitalization in patients with acute mania: a randomized controlled trial. *Bipolar disorders*, *20*(7), 614- 621.
68. Bravo, J. A., Forsythe, P., Chew, M. V., Escaravage, E., Savignac, H. M., Dinan, T. G., ... & Cryan, J. F. (2011). Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proceedings of the National Academy of Sciences*, *108*(38), 16050-16055.
69. Karimi, G., Sabran, M. R., Jamaluddin, R., Parvaneh, K., Mohtarrudin, N., Ahmad, Z., ... & Khodavandi, A. (2015). The anti-obesity effects of Lactobacillus casei strain Shirota versus Orlistat on high fat diet-induced obese rats. *Food & nutrition research*, *59*(1), 29273.
70. Núñez, I. N., Galdeano, C. M., de LeBlanc, A. D. M., & Perdigón, G. (2014). Evaluation of

immune response, microbiota, and blood markers after probiotic bacteria administration in obese mice induced by a high-fat diet. *Nutrition*, *30*(11-12), 1423-1432.

71. So, S. S., Wan, M. L., & El-Nezami, H. (2017). Probiotics-mediated suppression of cancer. *Current opinion in oncology*, *29*(1), 62-72.
72. Huang, L., Shan, Y. J., He, C. X., Ren, M. H., Tian, P. J., & Song, W. (2016). Effects of L. paracasei subp. paracasei X12 on cell cycle of colon cancer HT-29 cells and regulation of mTOR signalling pathway. *Journal of functional foods*, *21*, 431-439.
73. Liu, M. M., Li, S. T., Shu, Y., & Zhan, H. Q. (2017). Probiotics for prevention of radiation-induced diarrhea: A meta-analysis of randomized controlled trials. *PLoS One*, *12*(6), e0178870.
74. Reunanen, J., von Ossowski, I., Hendrickx, A. P., Palva, A., & de Vos, W. M. (2012). Characterization of the SpaCBA pilus fibers in the probiotic Lactobacillus rhamnosus GG. *Applied and environmental microbiology*, *78*(7), 2337-2344.
75. Arasu, M. V., Al-Dhabi, N. A., Ilavenil, S., Choi, K. C., & Srigopalram, S. (2016). In vitro importance of probiotic Lactobacillus plantarum related to medical field. *Saudi journal of biological sciences*, *23*(1), S6- S10.
76. Tytgat, H. L., Douillard, F. P., Reunanen, J., Rasinkangas, P., Hendrickx, A. P., Laine, P. K., ... & de Vos, W. M. (2016). Lactobacillus rhamnosus G G outcompetes Enterococcus faecium via mucus-binding pili: evidence for a novel and heterospecific probiotic mechanism. *Applied and environmental microbiology*, *82*(19), 5756-5762.
77. Siezen, R. J., Tzeneva, V. A., Castioni, A., Wels, M., Phan, H. T., Rademaker, J. L., ... & van Hylckama Vlieg, J. E. (2010). Phenotypic and genomic diversity of Lactobacillus plantarum strains isolated from various environmental niches. *Environmental Microbiology*, *12*(3), 758-773.
78. Holzapfel, W. H., Haberer, P., Geisen, R., Björkroth, J., & Schillinger, U. (2001). Taxonomy and important features of probiotic microorganisms in food and nutrition. *The American journal of clinical nutrition*, *73*(2), 365s-373s.
79. Martino, M. E., Bayjanov, J. R., Caffrey, B. E., Wels, M., Joncour, P., Hughes, S., ... & Leulier, F. (2016). Nomadic lifestyle of Lactobacillus plantarum revealed by comparative genomics of 54

strains isolated from different habitats. *Environmental microbiology*, *18*(12), 4974-4989.

80. Parente, E., Ciocia, F., Ricciardi, A., Zotta, T., Felis, G. E., & Torriani, S. (2010). Diversity of stress tolerance in Lactobacillus plantarum, Lactobacillus pentosus and Lactobacillus paraplantarum: a multivariate screening study. *International journal of food microbiology*, *144*(2), 270-279.
81. Jose, N. M., Bunt, C. R., & Hussain, M. A. (2015). Comparison of microbiological and probiotic characteristics of lactobacilli isolates from dairy food products and animal rumen contents. *Microorganisms*, *3*(2), 198-212.
82. Zago, M., Fornasari, M. E., Carminati, D., Burns, P., Suàrez, V., Vinderola, G., ... & Giraffa, G. (2011). Characterization and probiotic potential of Lactobacillus plantarum strains isolated from cheeses. *Food Microbiology*, *28*(5), 1033-1040.
83. Quatravaux, S., Remize, F., Bryckaert, E., Colavizza, D., & Guzzo, J. (2006). Examination of Lactobacillus plantarum lactate metabolism side effects in relation to the modulation of aeration parameters. *Journal of applied microbiology*, *101*(4), 903-912.
84. Ahrén, I. L., Xu, J., Önning, G., Olsson, C., Ahrné, S., & Molin, G. (2015). Antihypertensive activity of blueberries fermented by Lactobacillus plantarum DSM 15313 and effects on the gut microbiota in healthy rats. *Clinical Nutrition*, *34*(4), 719-726.
85. Chiu, Y. H., Lu, Y. C., Ou, C. C., Lin, S. L., Tsai, C. C., Huang, C. T., & Lin, M. Y. (2013). Lactobacillus plantarum MYL26 induces endotoxin tolerance phenotype in Caco- 2 cells. *BMC microbiology*, *13*(1), 1-9.
86. Ku, H. K., Lee, H., Choi, I. D., Ra, J. H., Kim, T. Y., Jeong, J. W., ... & Ahn, Y. T. A. (2014). 102: Immuno-stimulatory effect of Lactobacillus plantarum HY7712 via toll- like receptor 2 signaling pathway. *Cytokine*, *70*(1), 52.
87. Lee, J. J., Kwon, H., Lee, J. H., Kim, D. G., Jung, S. H., & Ma, J. Y. (2014). Fermented soshiho-tang with Lactobacillus plantarum enhances the antiproliferative activity in vascular smooth muscle cell. *BMC complementary and alternative medicine*, *14*(1), 1-8.
88. Kim, J. Y., Kim, H., Jung, B. J., Kim, N. R., Park, J. E., & Chung, D. K. (2013). Lipoteichoic acid isolated from Lactobacillus plantarum suppresses LPS-mediated atherosclerotic plaque

inflammation. *Molecules and cells*, *35*(2), 115-124.

89. Stevenson, C., Blaauw, R., Fredericks, E., Visser, J., & Roux, S. (2014). PP137-SUN: randomized clinical trial: effect of Lactobacillus plantarum 299V on symptoms of irritable bowel syndrome. *Clinical Nutrition*, (33), S71.
90. Wang, K., Li, W., Rui, X., Chen, X., Jiang, M., & Dong, M. (2014). Characterization of a novel exopolysaccharide with antitumor activity from Lactobacillus plantarum 70810. *International journal of biological macromolecules*, *63*, 133-139.
91. Ylönen, S. K., S. M. Virtanen, and Leif Groop. "The intake of potatoes and glucose metabolism in subjects at high risk for Type 2 diabetes." *Diabetic medicine: a journal of the British Diabetic Association* 24.9 (2007): 1049-1050.
92. Farnetti, Sara, et al. "Food fried in extra-virgin olive oil improves postprandial insulin response in obese, insulin-resistant women." *Journal of medicinal food* 14.3 (2011): 316- 321.
93. Dasa, Fikiru, and Tilahun Abera. "Why Fried Food is Unhealthy: Heat Induced Food Toxicants and Associated Health Risks." (2019).
94. Singh, Gitanjali M., et al. "Estimated global, regional, and national disease burdens related to sugar-sweetened beverage consumption in 2010." *Circulation* 132.8 (2015): 639-666.
95. Bhatia, Karanpreet, and Asmita Das. "Combinatorial drug therapy in cancer-New insights." *Life Sciences* (2020): 118134.
96. Kalathoti, Evangelina, et al. "Anticancer Potential of Cephalotaxine and Derivatives as an Alternative for Invasive Therapies." *Journal of Student Research* 10.2 (2021).
97. Luch, Andreas. "Nature and nurture–lessons from chemical carcinogenesis." *Nature Reviews Cancer* 5.2 (2005): 113-125.
98. King, Charles M. "N-Substituted aromatic compounds." *Chemical Carcinogenesis*. Springer, Boston, MA, 1982. 25-46.
99. Ward, Elizabeth. "Environmental and Occupational Carcinogens." *The American Cancer Society's Principles of Oncology: Prevention to Survivorship* (2018): 81-91.
100. Süss, R., Kinzel, V., & Scribner, J. D. (1973). A First Step: Elucidation of Tar Cancer. In *Cancer* (pp. 13-22). Springer, New York, NY.

101. Murata, M., Thanan, R., Ma, N., & Kawanishi, S. (2012). Role of nitrative and oxidative DNA damage in inflammation-related carcinogenesis. *Journal of Biomedicine and Biotechnology*, *2012*.

102. Duar, R. M., Lin, X. B., Zheng, J., Martino, M. E., Grenier, T., Pérez-Muñoz, M. E., ... & Walter, J. (2017). Lifestyles in transition: evolution and natural history of the genus Lactobacillus. *FEMS microbiology reviews*, *41*(Supp_1), S27-S48.

103. Sun, Z., Harris, H. M., McCann, A., Guo, C., Argimón, S., Zhang, W., ... & O'Toole, P. W. (2015). Expanding the biotechnology potential of lactobacilli through comparative genomics of 213 strains and associated genera. *Nature communications*, *6*(1), 1-13.

104. Zheng, J., Ruan, L., Sun, M., & Gänzle, M. (2015). A genomic view of lactobacilli and pediococci demonstrates that phylogeny matches ecology and physiology. *Applied and environmental microbiology*, *81*(20), 7233-7243.

105. Ventura, M., Turroni, F., Motherway, M. O. C., MacSharry, J., & van Sinderen, D. (2012). Host–microbe interactions that facilitate gut colonization by commensal bifidobacteria. *Trends in microbiology*, *20*(10), 467-476.

106. Michelini, S., Modesto, M., Pisi, A. M., Filippini, G., Sandri, C., Spiezio, C., ... & Mattarelli, P. (2016). Bifidobacterium eulemuris sp. nov., isolated from faeces of black lemurs (Eulemur macaco). *International journal of systematic and evolutionary microbiology*, *66*(3), 1567-1576.

107. Deo, P. (2021). The gut microbiome and use of pre and probiotics. *Outlet: New Zealand Stomal Therapy Nurses, The*, (Mar 2021), 16-17.

108. Liang, D., Leung, R. K. K., Guan, W., & Au, W. W. (2018). Involvement of gut microbiome in human health and disease: brief overview, knowledge gaps and research opportunities. *Gut pathogens*, *10*(1), 1-9

109. Wong, C. B., Odamaki, T., & Xiao, J. Z. (2019). Beneficial effects of Bifidobacterium longum subsp. longum BB536 on human health: Modulation of gut microbiome as the principal action. *Journal of functional foods*, *54*, 506-519.

110. Biavati, B. R. U. N. O., Whitman, W., Goodfellow, M., Kampfer, P., Busse, H. J., Trujillo, M. E., & Ludwig, W. (2012). Family I. Bifidobacteriaceae Stackebrandt, Rainey and Ward-Rainey

1997, 487 VP. In *Bergey's Manual of Systematic Bacteriology, Volume 5, The Actinobacteria* (p. 171). New York: Springer.

111. Turroni, F., Duranti, S., Bottacini, F., Guglielmetti, S., Van Sinderen, D., & Ventura, M. (2014). Bifidobacterium bifidum as an example of a specialized human gut commensal. *Frontiers in microbiology*, *5*, 437.

112. Turroni, F., Peano, C., Pass, D. A., Foroni, E., Severgnini, M., Claesson, M. J., ... & Ventura, M. (2012). Diversity of bifidobacteria within the infant gut microbiota. *PloS one*, *7*(5), e36957.

113. Vale, F. F., Vítor, J. M., & Oleastro, M. (2016). Probiotics as an Alternative Therapy for Helicobacter pylori-Associated Diseases. In *Helicobacter pylori Research* (pp. 543- 574). Springer, Tokyo.

114. Maynard, A. A., Dvorak, K., Khailova, L., Dobrenen, H., Arganbright, K. M., Halpern, M. D., ... & Dvorak, B. (2010). Epidermal growth factor reduces autophagy in intestinal epithelium and in the rat model of necrotizing enterocolitis. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *299*(3), G614-G622.

115. Jandú, J. J., Moraes Neto, R. N., Zagmignan, A., de Sousa, E. M., Brelaz-de-Castro, M. C., dos Santos Correia, M. T., & da Silva, L. C. (2017). Targeting the immune system with plant lectins to combat microbial infections. *Frontiers in pharmacology*, *8*, 671.

116. Schrader, K. D. (2010). *Investigating the control of Listeria monocytogenes on uncured, no-nitrate-or-nitrite-added meat products*. Iowa State University.

117. Mondal, S. B., Gao, S., Zhu, N., Liang, R., Gruev, V., & Achilefu, S. (2014). Real- time fluorescence image-guided oncologic surgery. *Advances in cancer research*, *124*, 171-211.

118. Boada, L. D., Henríquez-Hernández, L. A., & Luzardo, O. P. (2016). The impact of red and processed meat consumption on cancer and other health outcomes: Epidemiological evidences. *Food and Chemical Toxicology*, *92*, 236-244.

119. Daniel, C. R., Cross, A. J., Graubard, B. I., Hollenbeck, A. R., Park, Y., & Sinha, R. (2011). Prospective investigation of poultry and fish intake in relation to cancer risk. *Cancer prevention research*, *4*(11), 1903-1911.

120. Bocci, A., Sebastiani, B., Trotta, F., Federici, E., & Cenci, G. (2015). In vitro inhibition of 4-

nitroquinoline-1-oxide genotoxicity by probiotic Lactobacillus rhamnosus IMC501. *Journal of microbiology and biotechnology*, *25*(10), 1680-1686. Al-Henhena, N., Ying, R. P. Y., Ismail, S., Najm, W., Khalifa, S. A., El-Seedi, H., & Abdulla, M. A. (2014). Chemopreventive efficacy of Andrographis paniculata on azoxymethane-induced aberrant colon crypt foci in vivo. *PloS one*, *9*(11), e111118.

121. Pallem, P. V. S. P., Bodiga, S., & Bodiga, V. L. (2020). Dietary phytate lowers K-ras mutational frequency, decreases DNA-adduct and hydroxyl radical formation in azoxymethane-induced colon cancer. *Iranian journal of basic medical sciences*, *23*(1), 20.
122. Dominici, L., Villarini, M., Trotta, F., Federici, E., Cenci, G., & Moretti, M. (2014). Protective effects of probiotic Lactobacillus rhamnosus IMC501 in mice treated with PhIP. *Journal of microbiology and biotechnology*, *24*(3), 371-378.
123. Gratz, S., Mykkänen, H., & El-Nezami, H. (2005). Aflatoxin B1 binding by a mixture of Lactobacillus and Propionibacterium: in vitro versus ex vivo. *Journal of food protection*, *68*(11), 2470-2474.
124. Femia, A. P., Luceri, C., Dolara, P., Giannini, A., Biggeri, A., Salvadori, M., ... & Caderni, G. (2002). Antitumorigenic activity of the prebiotic inulin enriched with oligofructose in combination with the probiotics Lactobacillus rhamnosus and Bifidobacterium lactis on azoxymethane-induced colon carcinogenesis in rats. *Carcinogenesis*, *23*(11), 1953-1960.
125. Kim, K. N., Heo, S. J., Yoon, W. J., Kang, S. M., Ahn, G., Yi, T. H., & Jeon, Y. J. (2010). Fucoxanthin inhibits the inflammatory response by suppressing the activation of NF-κB and MAPKs in lipopolysaccharide-induced RAW 264.7 macrophages. *European journal of pharmacology*, *649*(1-3), 369-375.
126. Lee, H. A., Kim, H., Lee, K. W., & Park, K. Y. (2015). Dead nano-sized Lactobacillus plantarum inhibits azoxymethane/dextran sulfate sodium-induced colon cancer in Balb/c mice. *Journal of medicinal food*, *18*(12), 1400-1405.
127. Mohania, D., Kansal, V. K., Kruzliak, P., & Kumari, A. (2014). Probiotic Dahi containing

Lactobacillus acidophilus and Bifidobacterium bifidum modulates the formation of aberrant crypt foci, mucin-depleted foci, and cell proliferation on 1, 2- dimethylhydrazine-induced colorectal carcinogenesis in Wistar rats. *Rejuvenation research*, *17*(4), 325-333.

128. Dallal, M. M. S., Mojarrad, M., Baghbani, F., Raoofian, R., Mardaneh, J., & Salehipour, Z. (2015). Effects of probiotic Lactobacillus acidophilus and Lactobacillus casei on colorectal tumor cells activity (CaCo-2). *Archives of Iranian medicine*, *18*(3), 0- 0.
129. Rafter, J. (2002). Lactic acid bacteria and cancer: mechanistic perspective. *British Journal of Nutrition*, *88*(S1), S89-S94.
130. Burns, A. J., & Rowland, I. R. (2000). Anti-carcinogenicity of probiotics and prebiotics. *Current issues in intestinal microbiology*, *1*(1), 13-24.
131. Tojo, R., Suárez, A., Clemente, M. G., de los Reyes-Gavilán, C. G., Margolles, A., Gueimonde, M., & Ruas-Madiedo, P. (2014). Intestinal microbiota in health and disease: role of bifidobacteria in gut homeostasis. *World journal of gastroenterology: WJG*, *20*(41), 15163.
132. Kuugbee, E. D., Shang, X., Gamallat, Y., Bamba, D., Awadasseid, A., Suliman, M. A., ... & Shang, D. (2016). Structural change in microbiota by a probiotic cocktail enhances the gut barrier and reduces cancer via TLR2 signaling in a rat model of colon cancer. *Digestive diseases and sciences*, *61*(10), 2908-2920.
133. Ranjbar, S., Seyednejad, S. A., Azimi, H., Rezaeizadeh, H., & Rahimi, R. (2019). Emerging roles of probiotics in prevention and treatment of breast cancer: a comprehensive review of their therapeutic potential. *Nutrition and cancer*, *71*(1), 1-12.
134. Denev, S. A. (2006). Role of lactobacilli gastrointestinal ecosystem. *Bulgarian Journal of Agricultural Science*, *12*(1), 63.
135. Gosai, V., Ambalam, P., Raman, M., Kothari, C. R., Kothari, R. K., Vyas, B. R. M., & Sheth, N. R. (2011). Protective effect of Lactobacillus rhamnosus 231 against N-Methyl- N′-nitro-N-nitrosoguanidine in animal model. *Gut Microbes*, *2*(6), 319-325.
136. Ambalam, P., Raman, M., Purama, R. K., & Doble, M. (2016). Probiotics, prebiotics and colorectal cancer prevention. *Best practice & research Clinical gastroenterology*, *30*(1), 119-131.

137. Kim, Y. K., & Quadro, L. (2010). Reverse-phase high-performance liquid chromatography (HPLC) analysis of retinol and retinyl esters in mouse serum and tissues. In *Retinoids* (pp. 263-275). Humana Press, Totowa, NJ.